Asymmetry Pins Insutable

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During cell division, sibling cells of different developmental potentials can arise by the asymmetric localization of protein or RNA. A well-studied example is in the development of the fly peripheral nervous system, where the four cells of the sensory bristle arise from a single sensory organ precursor (SOP) through two rounds of asymmetric cell division. In the first round of division, the SOP divides to give a PIla cell and a PIlb cell. Numb and Prospero (Pros) are localized in the PIlb cell which then divides to give a tertiary progenitor PIII and a glial cell\textsuperscript{11}. PIII expresses high levels of Elav and low levels of Pros and divides to produce the neuron and sheath cells. The glial cell expresses low Elav and high Pros and is recognized by the marker Repo. This cell migrates away from the other cells of the lineage following differentiation. The PIla cell divides to give the hair and shaft cell. In loss-of-function numb mutants, the SOP divides symmetrically and gives two PIla cells, which do not produce neurons\textsuperscript{8,12} (Figure 1a).

Asymmetric division is also used in the development of the central nervous system. Most Drosophila neuroblasts divide along the apical–basal axis to give a large apical daughter cell and a smaller basal ganglion mother cell (GMC). This asymmetry is due to the localization of proteins such as Prospero and Numb in the basal cortical region of the neuroblast (NB). The apical daughter cell retains the NB character and continues to divide as a stem cell. The basal GMC, on the other hand, can give rise to either neurons or glial cells (Figure 1b).

**Insutable localization to the apical cortex is responsible for creating asymmetry**

The most important protein in the asymmetric division of NBs is Insutable (Insc), which has 859 amino acids and a SH3 domain\textsuperscript{5,6}. This protein is required for the localization of other proteins such as Pros and Miranda (Mir) which are responsible for asymmetric division. Drosophila NBs delaminate from a polarized epithelium in the ventral neuroectoderm and divide asymmetrically along their apical–basal axis. Ectodermal cells divide with the axis of their mitotic spindle parallel to the plane of the ectodermal layer. NBs divide with the axis of their

**Figure 1.** Division in (a) the peripheral nervous system; and (b) the central nervous system.
mitotic spindle perpendicular to the ectoderm. Insc is localized asymmetrically in the apical cortex of NBs during delamination as well as during the various stages of the cell cycle and becomes undetectable during anaphase. The localization of Insc to the apical pole of dividing neural precursors takes place before they enter prophase (Figure 2). Insc is also responsible for the correct orientation of the spindle axis in dividing delaminated NBs. As the cell cycle progresses, Insc-dependent apical localization of proteins such as Pros, Mir, Staufen (Stau) and Numb occurs before they are translocated to the basal pole.

**Insc maintains proper Pros localization**

Pros requires Insc for proper basal localization after a transient apical localization. Pros is a homeobox-containing transcriptional regulator which is required for proper neuronal differentiation, neuronal cell-fate specification, axon growth and guidance. Pros activates GMC-specific genes and represses genes which are normally expressed in NBs. pros RNA is found transiently localized in an apical crescent during late interphase and early prophase and is localized to the basal cortex during later prophase, metaphase, anaphase and telophase (Figure 2). In _inss_ mutants, Pros and _pros_ RNA show apical localization and no basal crescent is detectable.

**Stau localizes _pros_ RNA**

Stau, an RNA-binding protein has been identified in binding _pros_ RNA. It was identified by using _inss_ cDNA as a bait in a yeast two-hybrid screen. Stau is expressed in both GMCs and in NBs and is formed _de novo_ in GMCs. This was proved by showing that Stau levels were very low in newly formed GMCs. Stau localizes to the apical cortex during interphase and early prophase and is then localized asymmetrically to the basal cortex during late prophase, metaphase, anaphase and telophase. The role of Stau in localizing _pros_ RNA was discovered by the apical localization of _pros_ RNA in _stau_ mutants lacking both zygotic and maternal Stau contributions. Pros protein displayed normal basal localization in such mutants. Stau acts downstream of Insc because _stau_ mutants display only _pros_ RNA mislocalization, while _inss_ mutants display defects in Stau, Pros and _pros_ RNA localization as well as misorientation of mitotic spindles.

**Miranda – A multi-domain protein**

Mir, a multi-domain protein is required for the proper localization of Pros and Stau. It can also bind Insc. Mir too shows a transient apical localization during interphase and early prophase and then localizes to the basal cortex during the later stages of the cell cycle. Proper basal localization of Mir requires Insc. Loss-of-function _mir_ mutation causes cytoplasmic distribution of Pros and its segregation into both GMCs and NBs. It is seen that localization patterns of Pros and Numb are disrupted by actin-depolymerizing agents like Latrunculin A and B, but not by treatment with a microtubule depolymerizing agent like colcemid. This shows that microfilaments play a role in the movement of proteins from the apical to the basal cortical regions.

**Bazooka maintains apical localization of Insc**

Bazooka (Baz), a cytoplasmic protein with 2 PDZ domains which has overall sequence similarity to Par-3 of _C. elegans_ and rat ASIP has been shown to bind Insc and is responsible for its apical localization. In NBs, the spatial and temporal expression of Baz is similar to Insc. Both Baz and Insc localize to the apical cortex in the form of a crescent. In _baz_ mutants lacking both maternal and zygotic contributions of Baz, apical crescents of Insc are not formed, and diffuse staining of Insc is seen. A fully penetrant spindle-orientation defect is also seen. _baz_ mutant embryos also show _pros_ RNA localized all over the cell cortex. Despite the lack of crescent formation, Pros was found localized in the GMCs. Hence Baz is required only for the early steps of Pros localization which lead to the formation of basal crescents during metaphase. In contrast, the segregation of Pros into the budding GMC during anaphase and telophase appears to be controlled by a Baz-independent mechanism. Baz localization, like Insc, is dependent upon the cell cycle and is visible as a
crescent throughout the cell cycle and becomes indistinguishable during anaphase. A central domain of InsC called the asymmetric localization domain mediates direct interaction with Baz and is necessary for all aspects of the protein’s function and localization.\textsuperscript{7,16} Hence Baz recruits InsC to the cortical crescent, where InsC interacts with Stau and Mir and thus causes the localization of Pros and pros RNA to the basal cortical crescent.\textsuperscript{14,17}

**Pins interacts with InsC**

Pins (Partner of InsCutable) is a novel interactor which is shown to be responsible for the maintenance of InsC and Baz apical localization. The initial localization of InsC and Baz is independent of Pins, but these three proteins depend on each other for the maintenance of their apical character. Pins was identified by two independent methods—a yeast two-hybrid screen, where the asymmetric localization domain of InsC (amino acids 288–497) was used as the bait to screen a 0–21 h embryonic cDNA library\textsuperscript{18} and immunoprecipitation coupled to mass spectrometry.\textsuperscript{15} Pins has 658 amino acids, and has a molecular weight of 71,523 Daltons. It has 7 TPR (tetra-tricopeptide repeat) motifs at the N-terminal which are involved in general protein–protein interactions and is present in both epithelial cells and NBs. Pins also has three ‘GoLoco’ motifs which bind Gz and GzI subunits and may represent a novel Gz-binding motif. Immunoprecipitation studies show that InsC and Pins interact under in vivo conditions. Pins binds in vitro to both Gz and GzI (ref. 13). Using different segments of the InsC protein, it was deduced that the asymmetric localization domain of InsC interacts with Pins and that the N-terminal region of Pins is responsible for this interaction. Together these data suggest that InsC may act by recruiting Gz signalling proteins via Pins. As expected, it was seen that Pins localized to the apical crescent in NBs. Apical localization of Pins is seen only after delamination, whereas InsC forms an apical crescent during this process. This shows that Pins is not needed for the initial localization of InsC to the apical crescent, a process mediated by Baz. Pins crescents become more intense from prophase to anaphase of cell division, decrease during telophase and vanish after telophase. InsC and Pins are found colocalized after delamination.

In insc mutants, Pins is found distributed throughout the cortical region and is not asymmetric in distribution. Removal of zygotic and maternal Pros leads to the mislocalization of InsC and Baz in the embryo. Although the initial apical localization is normal, it cannot be maintained in the absence of Pins. baz mutants display abnormal localization of Pins and InsC. It is observed that pins loss-of-function displays a similar phenotype as insc loss-of-function mutation. It is characterized by the defective orientation of the mitotic spindle, by mislocalization of crescents of Mir, Pros, Numb and Partner of Numb (Pon). The phenotype differs slightly from that of insc loss-of-function in that the crescents showed a greater frequency of overlap with one of the poles. This shows that the coordination of mitotic spindle orientation with protein localization may be less disrupted in this mutant than in the loss-of-function of insc. Resolution of the distinct fates of the neurons RP2 and RP2sib formed from GMCs also frequently fails to occur. Often, duplicated RP2 neurons are found at the expense of RP2sib.

**Implications of the Pins discovery**

The pins loss-of-function phenotype shows that the initial localization of InsC to the apical crescent needs Baz and is independent of Pins. At later stages of the cell cycle, this mutant causes mislocalization of Baz, InsC, Stau, Pros, Numb and Pon, showing that Pins is needed for the maintenance of the localization of these proteins as opposed to their initial localization. Although no direct interaction has been shown between Pins and Baz, we know that Baz interacts with InsC which in turn interacts with Pins. The maintenance of the localization of Baz, InsC and Pins during later stages of the cell cycle is interdependent. The maintenance of apical-basal asymmetry may occur through the G-protein signalling cascade.

How Pins is necessary for the maintenance of Baz and InsC apical localizations is a mystery that remains to be solved. The interdependence of all the three interactors could suggest a mechanism involving some sort of complex necessary for the microfilament-dependent localization of the basal crescent elements. How these three apical interactors direct basal migration is a field open to speculation. Do G proteins play a role in the translocation of basal-crescent elements? If so, what is the downstream signalling pathway involved in this process? These are questions which still continue to interest researchers in the field of asymmetric cell division.


**ACKNOWLEDGEMENTS.** I am grateful to K. VijayRaghavan and Veronica Rodrigues for their valuable time, patience and help in bringing this article to its final form.

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